Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Review Article

Received on: 12-07-2015
Accepted on: 17-07-2015
Published on: 15-08-2015

Corresponding Author

*** P. Sridhar,**
Department of Pharmaceutical Analysis And Quality Assurance,
CMR College Of Pharmacy,
Kandlakoya (v),
Medchal road, Hyderabad – 501401, T.S, India.

Email: sridhar97045@gamil.com

Analytical Techniques by Mass spectrophotometry for the Estimation of Mirtazapinein Bulk and Pharmaceutical Dosage Forms: A Review

P. Sridhar*, C. Radhika, A. Ajitha, V. Uma Maheswara Rao

ABSTRACT

Mirtazapine (MRZ) has a piperazinoazepine structure1,2,3,4,10,14b-hexahydro-2- methylpyrazino [2,1-a]-pyrido [2,3-c][2] benzazepine}. It belongs to the class of noradrenergic and specific serotonergic inhibitor antidepressants (NaSSA). MRZ increases the central noradrenergic an serotonergic activity by blocking α2 adrenergic receptors. MRZ also acts as an antagonist of postsynaptic serotonin type 2 (5-HT2) and type 3 (5-HT3) drug has a different mechanism compared to most of the second generation antidepressants, and it is used to treat generalized anxiety , obsessive–compulsive , and post traumatic stress disorders . It has a high affinity for histamine H1 receptors: Literature survey reveals that Mirtazapine is estimated by Mass spectrometry.

Key-words: Mirtazapine, Mass spectrometry, MS instrumentation.

Cite this article as:
P. Sridhar, C. Radhika, A.Ajitha, V. Uma MaheswaraRao, Analytical Technique by Mass spectrophotometry for the Estimation of Mirtazapinein Bulk and Pharmaceutical Dosage Forms: A Review, Asian Journal of Pharmaceutical Technology & Innovation, 03 (13); 2015. www.asianpharmtech.com*

INTRODUCTION[1]

Mirtazapine is an atypical antidepressant drug usedin major depressive[1] disorder. It acts by potentiation ofnoradrenergic and serotonergic activity throughantagonism of á2 adrenergic autoreceptors andheteroreceptors.1 It also antagonises post-synapticserotonin type 2 (5-HT2) and type 3 (5-HT3) receptors.It is commercially available as racemic mixture of *S*(+)and *R*(-) enantiomers . The *S*(+) enantiomer isresponsible for blocking a2 activity and is more potent 5-HT2 antagonist whereas the *R*(-) enantiomer blocks 5-HT3 activity and contributes equally to the blocking of a2autoreceptors.2,3 Chemically, it is *R,S*-(1,2,3,4,10,14bhexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c]benzazepine). Mirtazapine is not considered to have a risk of many of the side effects often associated with other antidepressants like the SSRIs, and may actually improve certain ones when taken in conjunction with them.These adverse effects include decreased appetite, weight loss, insomnia, nausea and vomiting, diarrhoea, urinary retention, increased body temperature, excessive sweating, pupil dilation and sexual dysfunction.

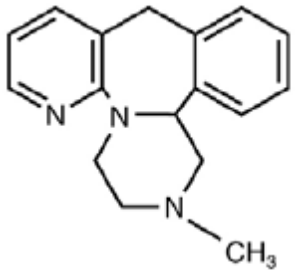

Fig no 1:Structure of Mirtazapine

Mass spectrometry (MS) has been described as the smallest scale in the world, not because of its size of what it weighs a molecule and a micro analytical technique that can be used selectively to detect and determine the amount of a given analyte . MS is also used to determine the elemental composition and some aspect of the molecular structure of an analyte. Unique features of MS include its capacity for direct determination of the nominal mass of an analyte, and to produce an detect fragments of the molecule that correspond to discrete groups of atoms of different elements . The tools of MS are mass spectrometers, and data are called mass spectra that can be displayed in many different ways, which allow the desired information about the analyte to be easily extracted. A MS is an apparatus which produces a beam of gaseous ions from a sample, sorts out the resulting mixture of ions according to their mass-to-charge ratios, and provides output signals which are measures of relative abundance of each ionic species present. MS are usually classified on the basis of how the mass separation is accomplished, but they all can be described as ion optical devices which separate ions according to their mass-to-charge (m/z) ratios by utilizing electric and/or magnetic force fields . The concept of MS is to form ions from a sample, to separate the ions based on their m/z ratio (this can be considered to be the same as the mass because the ion has only a single charge in most cases), and to measure the abundance of the ions. In modern MS instrumentation used in environmental analyses, all of the functions (ionization separation of the ions, rate of data acquisition, detection of the ions, and storage of the data) are under computer control. Gaseous molecules are ionized in the ion source to form molecular ions which some of that will fragment. By various processes, ions of differing m/z values pass through the mass analyzer one at a time to reach

the detector. When the ions strike the detector, they are converted into an electrical signal which, in turn, is converted into a digital response that can be stored by the computer . A mass spectrometer does not directly determine mass but, determines the mass of a molecule by measuring the m/z of its ion. The knowledge of the m/z of the ions enables one to determine what is present, while the measured ion intensities answer the question of how much is present. In addition, systematic interpretation of the mass spectra provides a detailed picture of the ionization process which, in turn, may be utilized in the elucidation of molecular structures. This definition of the term m/z is important to understanding of MS. It should be noted that the m/z value is a dimensionless number that is always used as an adjective, e.g. the ions with m/z 256, or the ion has an m/z value of 256. A recording of the number of ions (abundance) of a given m/z value as a function of the m/z value is a mass spectrum. The mass component that makes up the dimensionless m/z unit is based on an atomic scale rather than the physical scale normally considered as mass. Only ions are detected in mass spectrometer and any nonionic particles that have no charge are removed from the mass spectrometer by the continuous pumping that maintains the vacuum. The MS first must produce a collection of ions in the gas phase. These ions are separated according to their m/z values in a vacuum where the ions cannot collide with any other forms of matter during the separation process. Ions of individual m/z values are separated and detected in order to obtain the mass spectrum. Separation of ions in an evacuated environment is mandatory. If an ion collides with neutrals in an elastic collision during ion separation process, the ion‘s direction of travel could be altered and ion might not reach the detector. If an ion‘s collision with neutral is inelastic, sufficient energy transfer may cause it to decompose, meaning that the original ion will not be detected. Close encounters between ions of the same charge can be cause deflection in the path of each. Direct contact between ions of opposite charge sign will result in neutralization. Ions are positively or negatively charged atoms, groups of atoms, or molecules. The process whereby an electrically neutral atom or molecule becomes electrically charged, due to losing or gaining one or more of its extra nuclear electrons, is called ionization . Although both positive and negative ions can be analyzed by MS, the majority of instruments are used to investigate positive ions because in most ion sources they are produced in larger number than negative ions. There is a minimum amount of energy, characterized by the ―ionization potential,‖ that must be provided in order for ion formation to occur. The first ionization potential of an atom or molecule is defined as the energy input required removing (to infinite distance) a valence electron from the highest occupied atomic or molecular orbital of the neutral particle to form the corresponding atomic or molecular ion, also in its ground state. When only one electron is removed the ion is called an atomic or molecular ion; often the term ―parent ion‖ is used. The formation of parent ions may be considered as ionization without cleavage. The numerical magnitude of the ionization potential is influenced by such factors as the charge upon the nucleus, the atomic or molecular radius, the shielding effect of the inner electronic shells, and the extent to which the most loosely bound electrons penetrate the cloud of electric charge of the inner shells Because only ions can be detected in MS, any particles that are not ionic (molecules or radicals) are removed from the MS by the continuous pumping that maintains the vacuum. When only individual ions are present, they can be grouped according to their unique properties (mass and number of charges) and moved freely from one point to another. In order to have individual ions free from any other forms of matter, it is necessary to analyze them in a vacuum, which means that the ions must be in the gas phase. It is a fundamental requirement of MS that ions be in the gas phase before they can be

separated according to their individual m/z values and detected. Due to ionization sources such as electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), MS has become an irreplaceable tool in the biological sciences.Over the past decade, MS has undergone tremendous technological improvements allowing for its application to proteins, peptides, carbohydrates, DNA, drugs, and many other biologically relevant molecules .

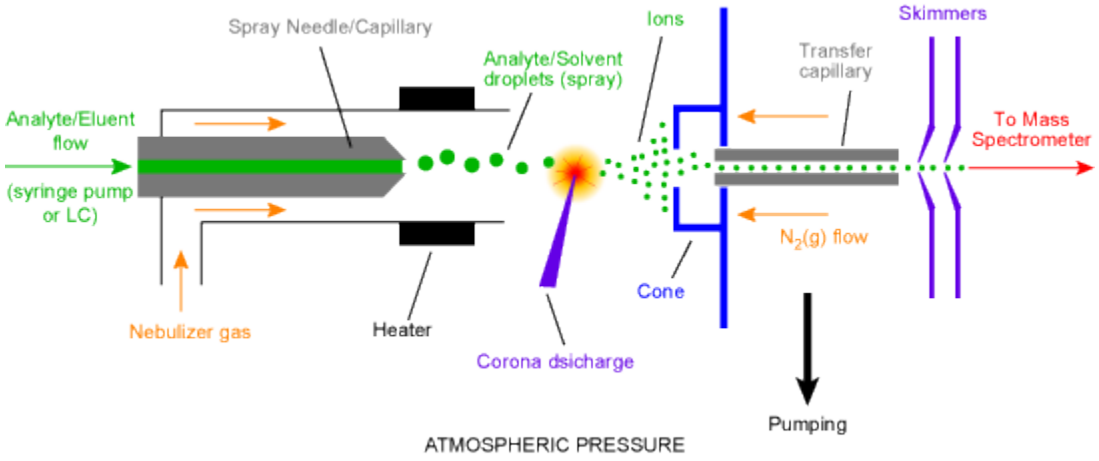

Fig No:2

INSTRUMENTATION OF MASS SPECTROMETRIC DETECTION:

Mass spectrometry is a particularly powerful scientific technique because it can be successfully applied even if you have only a tiny quantity available for analysis—as little as 10-12 g, 10-15 moles for a compound of mass 1000 Daltons (Da). Compounds can be identified through mass spectrometry at very low concentrations (one part in 1012) in chemically complex mixtures. The basic mass spectrometry processes of instrumentation are consisted of (1) introduction of sample; a sample which can be a solid, liquid, or vapor is loaded onto a mass spectrometry device and is vaporized, (2) ionization; sample components are ionized by one of several available methods to create ions, (3) analyzer sorting; the ions are sorted in 445 an analyzer according to their m/z ratios through the use of electromagnetic fields, (4) detector; the ions then pass through a detector where the ion flux is converted into a proportional electrical current and (5) date conversion; the magnitude of the ion/electrical signals is converted into a mass spectrum. MS instruments consist of three modules: an ion source, which can convert gas phase sample molecules into ions (or, in the case of ESI, move ions that exist in solution into the gas phase); a mass analyzer, which sorts the ions by their masses by applying electromagnetic fields; and a detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds .

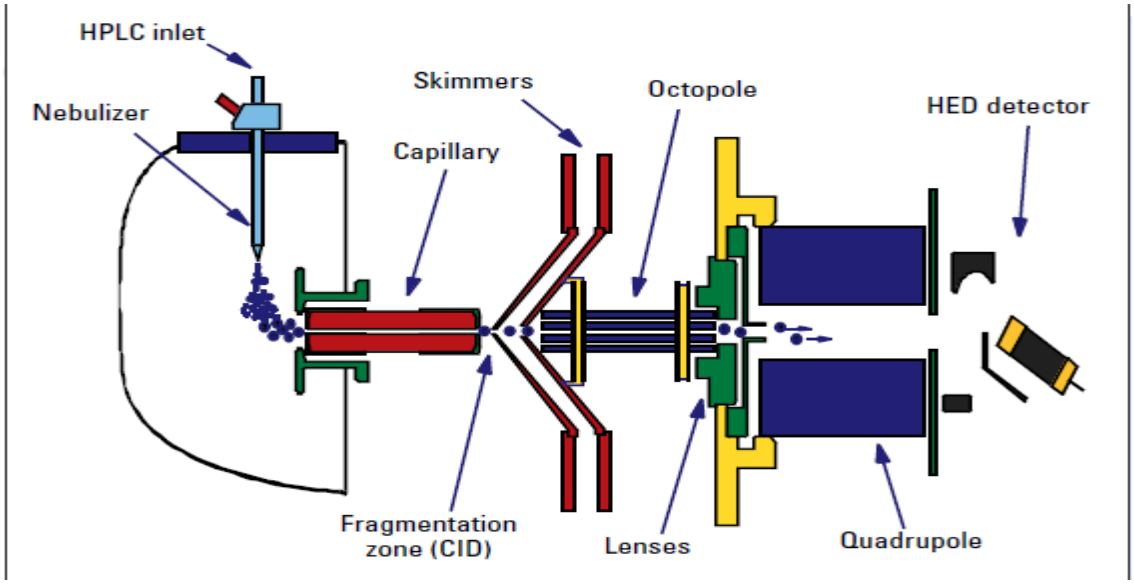

FIG NO 3 :LC/MSD System API interface

ANALYTICAL METHODOLOGIES:

GulshanBansal et al., Forced degradation study on mirtazapine is carried out in accordance with ICH guideline under the conditions ofhydrolysis, oxidation, dry heat and photolysis. A LC-MS compatible LC-UV method is developed for separation ofdegradation products and the drug. The drug was found stable to peroxide oxidative degradation at 30_C, hydrolyticdegradation at 80_C and thermal degradation at 50_C. The drug was also stable to photolytic degradation in dry
state and in alkaline medium. However, little degradation (less than 5%) was observed in neutral and acidicmedia under photolytic condition. A Major (DP-I) and three minor (DP-II to DP-III) products were separated inacidic photolytic sample. The DP-I and DP-II were detected in LC-MS. These were characterized as 2-Methyl-2-oxy-1,2,3,4,9,13b-hexahydro-2,4a,5-triaza-tribenzo[*a,c,e*]cyclohepten-11-ol and 4,5,10,14b-tetrahydro-1H-3,5a,6-triaza-dibenzo-[*a,d*] heptalen-12-ol, respectively on the basis on mass fragmentation data obtained through theirMS spectra. The DP-I is proposed to form due to photocatalyzed hydroxylation or aromatic rings and oxidation oftertiary amines while product DP-II was formed by N-oxidation followed by ring expansion.

Jun Wen et al., A simple, rapid and sensitive chiral capillary zone electrophoresis coupled with
acetonitrile-field-amplified sample stacking method was developed that allows thesimultaneous enantioselective separation of the mirtazapine, N-demethylmirtazapine,8-hydroxymirtazapine and mirtazapine-N-oxide. The separation was achieved on anuncoated 40.2 cm × 75 μM fused silica capillary with an applied voltage of 16 kV. Theelectrophoretic analyses were carried out in 6.25 mM borate–25 mM phosphate solution atpH 2.8 containing 5.5 mg/mL carboxymethyl-β-cyclodextrin. The detection wavelength was200 nm. Under these optimized conditions, satisfactory chiral separations of four pairenantiomers were achieved in less than 7 min *in vitro*. After one step clean-up liquid-liquidextraction using 96-well format, sample was introduced capillary zone electrophoresis withacetonitrile-field-

amplified sample stacking to enhance the sensitivity of enantiomers. Themethod was validated with respect to specificity, linearity, lower limit of quantitation,accuracy, precision, extraction recovery and stability. The lower limit of quantification was 0.5 ng/mL with linear response over the 0.5–50 ng/mL concentration range for eachmirtazapine, N-demethylmirtazapine and 8-hydroxymirtazapine enantiomer. The developedand validated method has been successfully applied to the enantioselective pharmacokineticstudies in 12 healthy volunteers after oral administration of rac- mirtazapine.

BhusariK.P et al.,This study describes the development and validation of stability indicating RP-HPLC method forMirtazapine, an antidepressant drug. In order to investigate the stability of drug, a stress testing of drugsample by exposing it to variety of forced degradation conditions has been recommended. Mirtazapinewas subjected to stress degradation1 under different conditions recommended by Internationalconference on Harmonization (ICH). The ICH guideline gives parameters to be considered whenvalidating methods, the objective of validation of an analytical procedure is to demonstrate that it issuitable for its intended purpose. Stress testing methods are screening methods to be used to understandthe degradation chemistry of a drug and therefore do not need to be validate to the extent of finalcontrol methods. The sample so generated was used to develop a stability indicating High Performanceliquid Chromatographic method for Mirtazapine. The chromatographic separation of Mirtazapine and
its degradation products was done on C18 column. The mobile phase containing mixture of Water andAcetonitrile in ratio 80:20 was found to be most satisfactory at a flow rate of 1mllmin. Detection wascarried out using single wavelength detector at 225nm. The retention time under optimizedchromatographic condition was found to be 8.43 minutes, with asymmetry of 1.50. A good linearresponse was observed in the range of 5-25ug/ml. The method showed good recoveries (average101.37).

Sk. Benajeer et al., Mirtazapine is chemically a tetratetracyclic (pyridodibenzazepine) derivative which is classified as anoradrenergic and specific serotonergic antidepressant (NaSSA). Mirtazapine acts as an antagonist at centralpre-synaptic α2-receptors, inhibiting negative feedback to the presynaptic nerve and causing an increase in NErelease. It is also used for the treatment of Posttraumatic stress disorder. In present work, simple, sensitive,accurate and economical spectroscopic method has been developed for the estimation of mirtazapine in bulk andits pharmaceutical dosage forms. An absorption maximum was found to be at 232nm with the solvent systemphosphate buffer (pH 6.8). The drug follows beer's law in the range of 5-30µg/ml with correlation coefficient of0.999. The percentage recovery of mirtazapine ranged from 99.57-100.26% in pharmaceutical dosage form.Results of the analysis were validated for accuracy, precision, LOD, LOQ and were found to be satisfactory.The proposed method is simple, rapid and suitable for the routine quality control analysis.

HodaLavasaniet al.,A rapid and sensitive HPLC method has been developed for the quantification of mirtazapine (MRZ), a noradrenergic and specific serotonergic inhibitor antidepressant (NaSSA) and its two major metabolites N-desmethyl mirtazapine (NDM) and 8-hydroxymirtazapine (8-OHM) in human plasma.The separation was achieved using Chromolith C_{18} column and a mobile phase of acetonitrile:

phosphate buffer (pH = 3, 20:80, v/v) in isocratic mode at a flow rate of 2 mL/min. A fluorescence detector was set at 290 and 350 nm for excitation and emission, respectively. Zolpidem was used as the internal standard. Liquid-liquid extraction was applied for sample clean up. All analytes were eluted in less than 5 minutes with LOQ of 1 ng/mL for MRZ and 2 ng/mL for both NDM and 8-OHM. The developed method was successfully applied to quantify MRZ and its metabolites in plasma of a healthy volunteer.

MarlusChorilli et al., Mirtazapine (MTZ) is an antidepressant drug, which belongs to the chemical class of piperazinoazepines. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the quantifica- tion of MTZ in plasma at the concentrations associated with therapy. Diazepam (DZP) was used as internal standard, added to 200 µL of plasma sample prior to a liquid-liquid extraction using hexane. Chroma- tographic separation was achieved on an Agilent® Eclipse XDB C-18 column (100 × 2.1 mm, 3.5 µm) in iso- cratic mode at 40°C. Mobile phase was 10 mM ammonium acetate/acetonitrile/formic acid (60/40/0.1, v/v/v) at a constant flow rate of 0.5 mL·min–1. The injection volume was 10 µL and the total run time was 3.2 min. The method shows selectivity and linearity. The detection and quantitation limits were established at 0.17 and 0.50 ng·mL–1, respectively. The extraction recoveries for MTZ and DZP were found to be between 84.9 and 93.9%. The intra-day and inter-day precision and accuracy fulfill at the international acceptance criteria. The method shows to be stable for the studied parameters. Therefore, a rapid, specific, and sensitive LC-MS/MS method for quantification of MTZ in human plasma was developed and can be used in therapeu- tic drug monitoring of this drug.

R. NageswaraRao et al., A simple and rapid reversed-phase high-performance liquidchromatographic method has been developed for the separationand simultaneous determination of related substances ofmirtazapine in bulk drugs and pharmaceutical formulations. Siximpurities, including one degradation product of mirtazapine, havebeen separated on a BDS Hypersil (4.6 × 250 mm; particle size 5µm) column with a mobile phase consisting of 0.3% triethylamine(pH 3.0)–acetonitrile (78:22 v/v) eluted in an isocratic mode andmonitored with a photo diode array detector at 215 nm. Thechromatographic behavior of all the analytes was studied undervariable compositions of different solvent systems, temperatures,buffer concentrations, and pH values. The method was validated interms of accuracy, precision, and linearity. The inter- and intra-dayassay precision was found to be < 0.98% [relative standarddeviation; (RSD)] and the recoveries were in the range95.54–102.22% with RSD < 2.21%. The correlation coefficientsfor calibration curves for mirtazapine as well as impurities were inthe range of 0.9941–0.9999. The method was successfully appliedto the analysis of commercial formulations and the recoveries ofmirtazapine were in the range of 99.38–100.73% with < 0.52%RSD. The method is useful not only for rapid evaluation of thepurity of mirtazapine, but also for the simultaneous determinationof related substances in bulk drugs and pharmaceuticalformulations.

Ney Carter Borges et al., In the present study a simple, fast, sensitive and robust method to quantify mirtazapine in human plasma using quetiapine as the internal standard (IS) is described. The analyte and the IS were extracted from human plasma by a simple protein precipitation with methanol and were

analyzed by high-performance liquid chromatography coupled to an electrospray tandem triple quadrupole mass spectrometer (HPLC-ESI-MS/MS). Chromatography was performed isocratically on a C_{18}, 5 μm analytical column and the run time was 1.8 min. The lower limit of quantitation was 0.5 ng/mL and a linear calibration curve over the range 0.5–150 ng/mL was obtained, showing acceptable accuracy and precision. This analytical method was applied in a relative bioavailability study in order to compare a test mirtazapine 30 mg single-dose formulation vs a reference formulation in 31 volunteers of both sexes. The study was conducted in an open randomized two-period crossover design and with a 14 day washout period. Since the 90% confidence interval for C_{max}, AUC_{last} and AUC_{0-inf} were within the 80–125% interval proposed by the Food and Drug Administration and ANVISA (Brazilian Health Surveillance Agency), it was concluded that mirtazapine 30 mg/dose is bioequivalent to the reference formulation, according to both the rate and extent of absorption. Copyright © 2012 John Wiley & Sons, Ltd.

L Labaţ et al., Four analytical methods have been developed for the quality control of tablets containing mirtazapine: spectrophotometry, spectrofluorimetry, high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). All the methods only require a simple extraction procedure of mirtazapine from the tablets before analysis. The concentration of mirtazapine in solutions was determined in the linearity range of 5–25 μg/ml at λ=315 nm for spectrophotometry and at λ=220 nm for HPLC and CZE. Spectrofluorimetric determinations were achieved at $\lambda_{excitation}$=328 nm and $\lambda_{emission}$=415 nm in the linearity range of 2–25 ng/ml. All the methods gave similar results and were validated for selectivity, linearity, precision and sensitivity. Spectrometric methods gave slightly higher RSD values (up to 2.54%). The four methods were directly and easily applied to the pharmaceutical preparation with accuracy, resulting from recovery experiments between 99.72% in HPLC and 101.47% in spectrofluorimetry.

Reference:

1. AmanAnand, GulshanBansal, LC-UV and LC-MS studies on mirtazapine to characterize its degradationProducts, J Pharm Educ Res Vol. 2, Issue No. 1, June 2011.
2. Jun Wen 1, Wen-Ting Zhang 1, Wei-Qun Cao, Ji Li , Fang-Yuan Gao , Enantioselective Separation of Mirtazapine and Its Metabolitesby Capillary Electrophoresis with Acetonitrile Field-AmplifiedSample Stacking and Its Application, *Molecules* 2014, *19*, 4907-4923; doi:10.3390/molecules19044907.
3. Bhusari K.P, Tajne M.R. and Ahmed R.H. Stress Degradation Studies and Development of Validated StabilityIndicating Method for Assay of Mirtazapine,Res.J.Chem.Sci.Research Journal of Chemical SciencesVol. **1(4),** 74-79, July **(2011).**
4. Sk. Benajeer, K. Venkataramana, V.Kotireddy, New Simple UV SpectrophotometricMethod for Determination of Mirtazapinein Bulk and pharmaceutical dosage forms,ISSN : 0975-9492 Vol 3 No 10 Oct 2012.

5. HodaLavasani, Mario Giorgi, BehjatSheikholeslami,A Rapid and Sensitive HPLC-Fluorescence Method for Determination of Mirtazapine and Its two Major Metabolites in Human Plasma ,Iranian Journal of Pharmaceutical Research (2014), 13 (3): 853-862.
6. Marlus Chorilli1, Rudy Bonfilio1, Caroline RossimLouvandini,Development and Validation of an LC-MS/MS Method for Quantitative Analysisof Mirtazapine in Human Plasma, *American Journal of Analytical Chemistry*, 2011, 2, 650-657.
7. R. NageswaraRaoand A. NarasaRaju, Development and Validation of a Reversed-PhaseHPLC Method for Separation and SimultaneousDetermination of Process-Related Substances ofMirtazapine in Bulk Drugs and Formulations,Journal of Chromatographic Science, Vol. 47, March 2009.
8. Ney Carter Borges, Rafael EliseoBarrientos-AstigarragaA fast, sensitive and simple method for mirtazapine quantification in human plasma by HPLC-ESI-MS/MS. Application to a comparative bioavailability study, Biomedical ChromatographyVolume 26, Issue 11, pages 1399–1407, November 2012.
9. L Labat, P Dallet, E Kummer, J.P Dubost, Spectrophotometric, spectrofluorimetric, HPLC and CZE determination of mirtazapine in pharmaceutical tabletsJournal of Pharmaceutical and Biomedical Analysis,Volume 28, Issue 2, 15 April 2002, Pages 365–371.
10. Maris FA, Dingler E and Niehues S. High-performance liquid chromatographic assay with fluorescence detection for the routine monitoring of the antidepressant mirtazapine and itsdemethyl metabolite in human plasma. *J. Chromatogr. B Biomed. Sci. Appl*. (1999) 721: 309-316.
11. Morgan PE, Tapper J and Spencer EP. Measurement of total mirtazapine and normirtazapine in plasma/serum by liquid chromatography with fluorescence detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. (2003) 798: 211-215.
12. Mercolini L, Mandrioli R, Saladini B, Conti M, Baccini C and Raggi MA. Quantitative analysis of cocaine in human hair by HPLC with fluorescence detection. *J. Pharm. Biomed. Anal*. (2008) 48: 456-461.
13. de Santana FJ and Bonato PS. Enantioselective analysis of mirtazapine and its two major metabolites in human plasma by liquid chromatography-mass spectrometry after three-phase liquid-phase microextraction. Anal. Chim. Acta. (2008) 606: 80-91.
14. Kirchherr H and Kühn-Velten WN. Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. J. Chromatogr. B Analyt. Technol. Biomed. Life *Sci*. (2006) 843: 100-113.